
DIGITAL ACCESS TO SCHOLARSHIP AT HARVARD
DASH.HARVARD.EDU


HARVARD LIBRARY
Office for Scholarly Communication

High-Dose Mannose-Binding Lectin Therapy for Ebola Virus Infection

Citation
Michelow, Ian C., Calli Lear, Corinne Scully, Laura I. Prugar, Clifford B. Longley, L. Michael Yantosca, Xin Ji, et al. 2010. High-dose mannose-binding lectin therapy for ebola virus infection. Journal of Infectious Diseases 203(2): 175-179.

Published Version
doi:10.1093/infdis/jiq025

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:8646758

Terms of Use
This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. Submit a story .

Accessibility

High-Dose Mannose-Binding Lectin Therapy for Ebola Virus Infection

Ian C. Michelow,[1] Calli Lear,[2] Corinne Scully,[2] Laura I. Prugar,[2] Clifford B. Longley,[3] L. Michael Yantosca,[1] Xin Ji,[4] Marshall Karpel,[1] Matthew Brudner,[1] Kazue Takahashi,[1] Gregory T. Spear,[4] R. Alan B. Ezekowitz,[1] Emmett V. Schmidt,[5] and Gene G. Olinger[2]

[1]Programs of Developmental Immunology, Department of Pediatrics, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; [2]US Army Medical Research Institute of Infectious Diseases; Virology Division, Fort Detrick, Frederick, Maryland; [3]Enzon Pharmaceuticals, Bridgewater, New Jersey; [4]Department of Immunology and Microbiology, Rush University Medical Center, Chicago, Illinois; and [5]Cancer Research Center at Massachusetts General Hospital, Boston, Massachusetts

Mannose-binding lectin (MBL) targets diverse microorganisms for phagocytosis and complement-mediated lysis by binding specific surface glycans. Although recombinant human MBL (rhMBL) trials have focused on reconstitution therapy, safety studies have identified no barriers to its use at higher levels. Ebola viruses cause fatal hemorrhagic fevers for which no treatment exists and that are feared as potential biothreat agents. We found that mice whose rhMBL serum concentrations were increased ⩾7-fold above average human levels survived otherwise fatal Ebola virus infections and became immune to virus rechallenge. Because Ebola glycoproteins potentially model other glycosylated viruses, rhMBL may offer a novel broad-spectrum antiviral approach.

Circulating mannose-binding lectin (MBL) is a first-line host defense against a wide range of viral and other pathogens. MBL is a C-type lectin that recognizes hexose sugars including mannose, glucose, fucose, and *N*-acetylglucosamine on the surface of many pathogens. It does not recognize the terminal carbohydrates galactose and sialic acid on normal host cells. Therefore, MBL preferentially recognizes glycosylated viruses including influenza virus, human immunodeficiency virus, severe acute respiratory syndrome coronovirus (SARS-CoV), Ebola virus, and Marburg virus. It also recognizes many glycosylated gram-positive and gram-negative bacteria [1, 2]. As a result of common genetic variants, MBL serum levels in humans range from 0 to 10,000 ng/mL. Thirty percent of the human population has levels <500 ng/mL, which are associated with increased susceptibility to infections in children and immunocompromised individuals [3].

We previously reported preclinical studies that addressed the potential utility of recombinant human MBL (rhMBL) reconstitution therapy. MBL-knockout mice are highly susceptible to several bacteria including *Staphylococcus aureus* [1]. RhMBL improved survival in MBL-null mice to approximate survival among infected wild-type mice at doses that reconstituted the complement-activating capacity of MBL-knockout serum to a level comparable to that of wild-type mouse serum [1]. Doses of plasma-derived MBL and rhMBL designed to increase MBL concentrations to physiologic levels (>1000 ng/mL) in MBL-deficient humans were safe in early trials and did not elicit antibodies [3–5]. In contrast, although MBL replacement therapy enhanced opsonophagocytic potential, higher levels of plasma-derived MBL were needed to achieve MBL-mediated complement activation comparable to healthy controls [6], suggesting that above-replacement dosing will need attention.

Ebola and Marburg viruses of the filovirus family are among the most virulent causes of the human viral hemorrhagic fevers and cause devastating epidemics of fulminant and rapidly fatal disease. They constitute important biological threat agents because of their high mortality rates, capacity for large-scale dissemination, and potential for causing social disruption. Currently, there are no US Food and Drug Administration–approved therapeutic agents available to prevent or treat these lethal viral infections. Filovirus surface glycoproteins (GPs) are heavily glycosylated and contain high-mannose. As a result, MBL binds to Ebola and Marburg viruses and mediates complement-dependent virus neutralization [2]. Importantly, their surface glycoprotein structures are characteristic of a broad group of viruses in which *N*-linked glycosylation contributes to viral virulence [7]. Reasoning that MBL treatment is likely to be safe at supraphysiological levels, we evaluated an in vivo Ebola virus model to explore the possibility of using MBL as an immunotherapeutic agent. Our results showed that supraphysiological doses of MBL rescued ~40% of mice from lethal challenges when administered pre– or post–Ebola virus exposure.

Received 9 June 2010; accepted 14 October 2010.

Reprints or correspondence: Emmett V. Schmidt, Cancer Research Center at Massachusetts General Hospital, Boston, Massachusetts 02114 (eschmidt@partners.org).

The Journal of Infectious Diseases 2011;203:175–179

© The Author 2011. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/2.5), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

1537-6613/2011/2032-0001$15.00
DOI: 10.1093/infdis/jiq025

This novel paradigm suggests that high-dose MBL should be evaluated more broadly as an immunotherapeutic agent for a wide spectrum of glycosylated pathogens.

MATERIALS AND METHODS

Production and pharmacokinetics of rhMBL

Commercial-grade rhMBL was provided by Enzon Pharmaceuticals [8]. Human MBL concentrations and complement cleavage activity were measured as described elsewhere [9]. Pharmacokinetics of rhMBL concentration–time data were evaluated using noncompartmental modeling with WinNonlin Professional Edition (version 5.2; Pharsight). The area under the curve from zero to infinity ($AUC_{0-\infty}$) values were calculated using the linear trapezoidal method.

Murine Ebola model

We used a validated lethal Ebola Zaire mouse model developed at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) [10], with a double plaque-purified, mouse-adapted, Ebola isolate, EZ'76 Mp3 Vp2 Mp9 GH. The virus was inoculated intraperitoneally (i.p.) at 100 pfu ($3000 \times LD_{50}$) producing uniformly lethal disease in C57B6 mice using biosafety level-4 facilities. Research was conducted in compliance with the Animal Welfare Act and federal regulations in a fully accredited facility. To assess the effect of rhMBL on virus lethality, we treated Ebola virus–infected C57B6 mice i.p. with either 4.3 mg/kg or 20 mg/kg of rhMBL twice daily ~12 hours apart for 10 days. On the day of virus exposure, mice were treated and exposed to 100 pfu of mouse-adapted Ebola Zaire either 12 hours before or 1 hour after the first dose of rhMBL as indicated in Figure 1.

Mice were assessed daily for changes in physical appearance and weight. Viremia was assessed by reverse transcription-polymerase chain reaction (RT-PCR) and plaque assays as described elsewhere [11], and anti–Ebola virus antibodies were measured using standard enzyme-linked immunosorbent assays (ELISAs) [12]. Standard blood counts were evaluated with a Coulter A^C·T diff (Beckman Coulter). For analysis with flow cytometry, spleens were ground into single cell suspensions with the BD Medimachine tissue grinder. After incubation with Fc Block (BD), cells were washed and incubated with antibody (CD3 FITC BD no. 555274, CD8 V450 BD no. 560469, CD14 PerCP eBio no. 45-0141, CD4 PE eBio no. 12-0041-82, CD11b APC BD no. 553312, and CD19 PE-Cy7 BD no. 557655). Cells were washed with PBS and fixed in BD cytofix. Data were immediately acquired with a BD FACSCantoII and analyzed with FlowJo (version 7). The Bio-Plex Mouse Cytokine 23-Plex Panel assay (Bio-Rad 171-F11241) was used to measure multiple cytokines, chemokines, and growth factors in serum and tissue supernatants according to the manufacturer's instructions. Mice that survived the initial infection were tested for Ebola-specific serological response on day 21 and rechallenged with the same virus dose without further treatment, and antibody titers were retested 28 days later.

RESULTS

We previously found that rhMBL bound Ebola (Zaire) and Marburg (Musoke) envelope GPs [2]. RhMBL effectively blocked Ebola GP interactions with DC-SIGN, and HIV particles lacking gp120/gp41 pseudotyped with Ebola or Marburg GPs were neutralized by the lectin complement pathway [2]. To develop an in vivo test of rhMBL effectiveness, we determined that 100 ng/mL of rhMBL was the minimum concentration needed to inhibit ⩾90% infectivity of HepG2 cells using Ebola GP pseudotyped lentiviral particles and to inhibit ⩾90% infectivity of Vero E6 cells using recombinant Ebola Zaire virus (Mayinga strain)-eGFP (data not shown). We had previously found that a single intraperitoneal dose of 75 μg of rhMBL reconstituted the lectin complement pathway in MBL-knockout mice [1]. We compared the pharmacokinetic parameters (Table 1) of that single reconstitution dose (4.3 mg/kg) with a higher single intraperitoneal dose of 350 μg (20 mg/kg) to identify a potentially supraphysiological dose to test in model infections. The average maximum serum concentration (C_{max}) of both doses exceeded the minimum concentration of MBL that inhibited infection in vitro by at least 55-fold. The average ratio of maximum to baseline complement component 4 cleavage activity was 1.7 for the 75-μg rhMBL dose and 5.4 for the 350-μg dose.

Intraperitoneal administration of 100 pfu of native Ebola Zaire virus ($3000 \times LD_{50}$) is uniformly fatal in mice. Treatment with 75 μg of rhMBL per dose every 12 hours failed to protect mice from that virus inoculum. Therefore, we increased rhMBL to 350 μg administered every 12 hours for 10 days starting either 1 hour before or 12 hours after Ebola virus challenge (Figure 1*A* and 1*B*). When treatment was started 1 hour before virus infection, the supraphysiological dose increased survival to > 40% of mice in several trials (Figure 1*A*). We then started treatment 12 hours after viral infection. We compared survival in wild-type and complement component 3 (C3)–deficient mice as the inhibitory effects of MBL on Ebola virus are mediated by complement in cell culture [2]. Once again we saw an increase in survival from 0% to >40% in rhMBL-treated mice, and survival was dependent on an intact complement pathway, since C3-deficient mice did not survive (Figure 1*B*). All inoculated mice showed signs of infection according to standardized observation scores and weight loss, and surviving mice had detectable Ebola virus–specific antibodies 28 days after infection (data not shown).

We monitored the effect of treatment started 12 hours after infection on a variety of laboratory indices. Mean white blood cell counts were 9100 cells/mL in MBL-treated mice (n = 5)

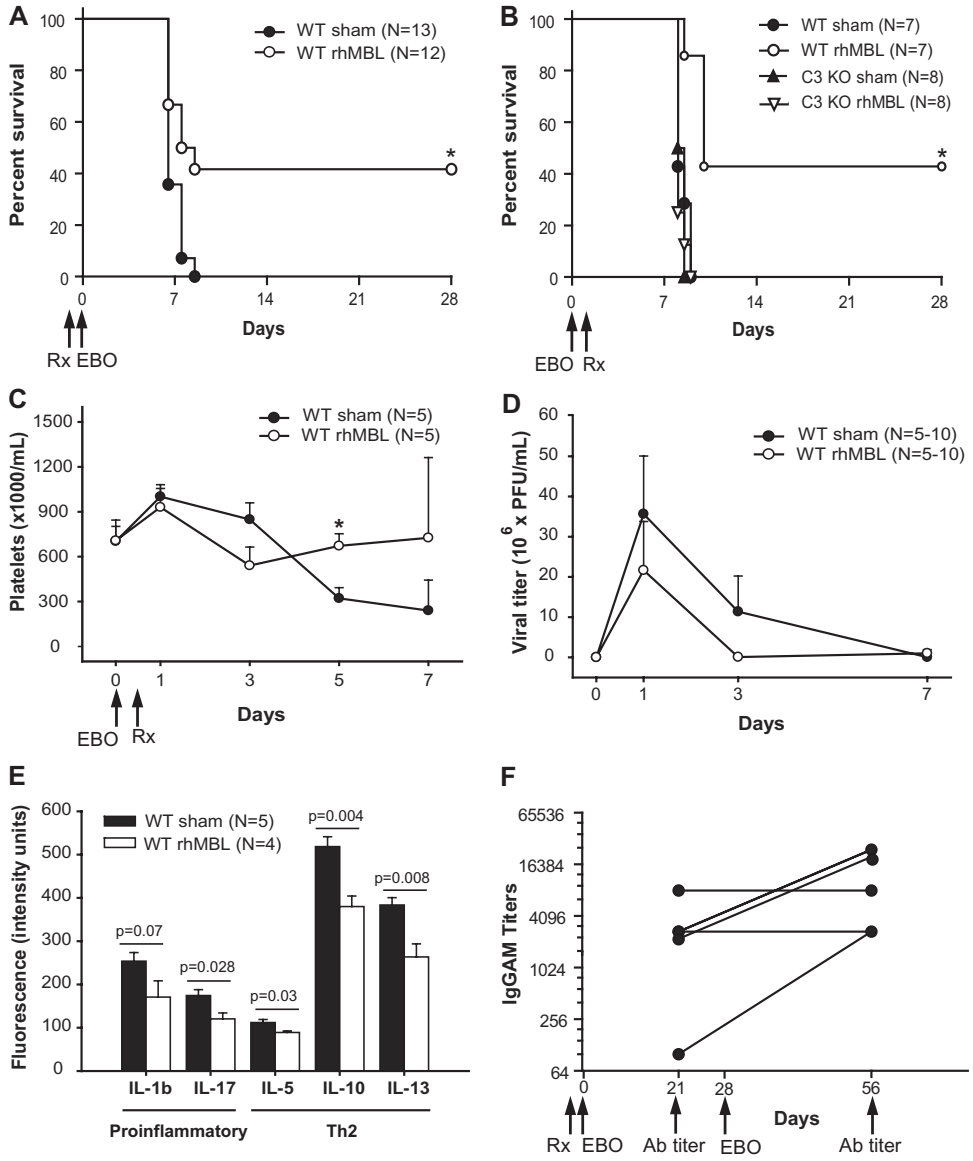

Figure 1. Survival and laboratory indices of filovirus-infected mice treated with recombinant human mannose-binding lectin (rhMBL). (*A*) Mouse survival when treated with rhMBL before Ebola virus inoculation. Sham-treated wild-type mice were compared with wild-type mice receiving 350 μg of rhMBL (referred to as Rx) administered intraperitoneally (i.p.) every 12 hours starting 1 hour before mouse-adapted Ebola virus (EBO) challenge (100 pfu). Shown is a Kaplan-Meier probability curve for mouse survival at the indicated times (*log-rank Mantel-Cox test, $P = .0075$). (*B*) Mouse survival when treated with rhMBL after Ebola virus inoculation. Sham-treated mice were compared with mice receiving 350 μg of rhMBL administered i.p. every 12 hours starting 12 hours after mouse-adapted Ebola virus challenge (100 pfu). Both wild-type (WT) and knockout mice lacking complement component 3 (C3 KO) were compared. Shown is a Kaplan-Meier probability curve for mouse survival at the indicated times (*log-rank analyses; WT: sham-treated vs rhMBL-treated, $P = .0013$; rhMBL-treated: WT versus C3 KO, $P = .0003$). (*C*) Platelet count analyses. RhMBL-treated mice had significantly lower platelet counts on day 5 after Ebola virus inoculation than sham-treated mice (*$P = .014$). (*D*) Viral plaque assays. RhMBL-treated mice tended to have lower viral titers than sham-treated mice but the differences were not statistically significant. (*E*) Intrahepatic cytokine responses. RhMBL-treated mice had lower proinflammatory and T helper cell type 2 (Th2) cytokine titers in liver homogenates on day 5 after inoculation (*P* values as shown). (*F*) Anti–Ebola virus titers in mice surviving Ebola virus infection. Fifteen wild-type mice received a 10-day course of rhMBL administered every 12 hours that was started 1 hour before inoculation with 100 pfu of mouse-adapted Ebola virus as indicated. Antibody (Ab) titers were obtained on day 21 and again on day 56. Mice were rechallenged with Ebola virus on day 28. The reciprocals of anti–Ebola virus antibody titers in 5 mice successfully treated with rhMBL are shown on the indicated days after initial and repeat challenges with Ebola virus.

compared with 4525 cells/mL on day 7 after infection in the surviving sham-treated mice (n = 4). Average lymphocyte counts were also higher in MBL-treated mice compared with controls (5500 cells/mL vs 2800 cells/mL, respectively). A similar trend was seen for platelet counts, which averaged 726,000 cells/mL in the treatment group and 239,000 cells/mL in the controls.

Table 1. Pharmacokinetic Parameters of Low- vs High-Dose Recombinant Human Mannose-Binding Lectin (rhMBL) Therapy in Uninfected Mice

	rhMBL		
Pharmacokinetic Parameter	75 μg (n = 5)	350 μg (n = 5)	*P* value
Maximum serum concentration (C_{max}, μg/ml)	5.9 (1.1)	17.1 (3.8)	.024
Half-life ($t_{1/2}$, hours)	12.6 (1.6)	14.9 (1.9)	.4
Area under the curve ($AUC_{0-\infty}$, hours·μg/ml)	123 (22)	301 (45)	.007
Time to maximum serum concentration (T_{max}, hours)	2.8 (.9)	2.1 (.7)	.6

NOTE. RhMBL was administered by a single intraperitoneal injection. Data are arithmetic mean (±SEM). Statistical differences were analyzed with the Student *t*-test (2-tailed). A value of $P < .05$ was considered to indicate a statistically significant difference. rhMBL, recombinant human mannose-binding lectin.

These differences were statistically significant for platelet counts on day 5 (672,000 cells/mL vs 322,000 cells/mL, $P = .014$; Figure 1*C*).

In a separate experiment, spleens were harvested on day 5 after infection (4 sham-treated and 4 MBL-treated mice). Constituent cell populations were assayed by flow cytometry. Numbers of splenic $CD3^{-}CD19^{+}$ cells (B lymphocytes) and $CD11b^{+}$ granulocytes were higher in MBL-treated mice (89.2% vs 85.1%, $P = .019$; 17.6% vs 12.8%, $P = .04$, respectively). The RNA viral loads as determined by RT-PCR in blood, liver, and spleen 5 days after infection were similar in sham- and rhMBL-treated mice ($P > .05$). Virus titers in blood were generally lower on days 1 and 3 in rhMBL-treated mice as determined by plaque assays ($P > .05$; Figure 1*D*). Of 23 cytokines and chemokines tested in serum, liver, and spleen on day 5 after inoculation, lower values (fluorescence intensity units) for interleukin (IL)-1b (170 vs 253, $P = .07$), IL-5 (89 vs 112, $P = .03$), IL-10 (379 vs 518, $P = .004$), IL-13 (264 vs 384, $P = .008$), and IL-17 (120 vs 174, $P = .028$) were found in liver homogenates from rhMBL-treated mice (Figure 1*E*). We tested protective immunity in 5 seropositive mice that survived initial infection by rechallenging them with native Ebola virus 28 days after initial infection. It is noteworthy that all MBL-treated survivors also survived the second viral challenge. Similar or higher immunoglobulin G, A, and M antibody titers were seen 28 days after the second challenge with the virus (Figure 1*F*).

DISCUSSION

In the past 3 decades, approved antivirals have increased from a few nucleoside analogues to well over 40 drugs [13]. The human immunodeficiency virus (HIV) and hepatitis C virus (HCV) epidemics particularly drove antiviral discovery toward rationally designed drugs targeting specific viral enzymes. Although this approach was remarkably effective, the advent of newly emerging or drug-resistant viruses that threaten humans calls for the development of more broadly active agents targeting viral components shared among viruses. *N*-glycosylation of viral envelopes is an important such target shared between influenza, HIV, HCV, West Nile virus, SARS-CoV, Hendra virus, Nipah virus, and filoviruses (Ebola and Marburg viruses) [7]. To assess one possible strategy against *N*-glycosylated viruses, we tested a stringent Ebola virus infection model ($3000 \times LD_{50}$) in mice.

Filovirus infections are characterized by marked lymphopenia, severe degeneration of lymphoid tissues, dysregulated dendritic cell function, and cytokine storms—all hallmarks of pathogens that subvert both innate and adaptive immune responses [14]. Nevertheless, survivors exhibit detectable virus-specific antibody responses [15]. Therefore, we hypothesized that administration of a recombinant innate immune molecule that targets glycosylated viruses might bridge an infected individual to recovery. Here we show for the first time that rhMBL can be used as a therapeutic agent to achieve serum concentrations in mice that correspond to levels in humans that are 7–24-fold higher than average human concentrations and complement cleaving activity that is >5-fold higher than baseline values in mice. This result confirms our previous in vitro data showing that MBL possesses complement-dependent intrinsic antimicrobial activity [2].

Biological responses of the infected mice to rhMBL treatment further indicated that our strategy targeted the main pathogenic effects of Ebola viruses. MBL-treated mice had higher B lymphocyte and $CD11b^{+}$ granulocyte counts and demonstrated down-regulation of intrahepatic proinflammatory (IL-1b and IL-17) and Th2 cytokines (IL-5, IL-10, and IL-13) early in the course of infection (Figure 1*E*), suggesting that rhMBL may mitigate the detrimental effects of the characteristic cytokine storm. MBL-treated mice tended to have greater inhibition of viral replication on days 1 and 3 after infection ($P > .05$; Figure 1*D*). Most important, rhMBL treatment bridged surviving mice to development of an effective adaptive immune response (Figure 1*F*). Future experiments will be needed to scale high-dose rhMBL therapy for use in larger animal models and to test rhMBL in combination with other promising experimental therapies such as small molecule inhibitors, coagulation modulators, antisense technologies, therapeutic antibodies and

cytokines, and postexposure vaccination. In summary, we report that supraphysiologic rhMBL therapy may be an effective immunotherapeutic strategy against Ebola virus, and since Ebola glycoproteins potentially model other glycosylated viruses, rhMBL therapy may offer a novel broad-spectrum antiviral approach.

Funding

This study was supported by grant U01-AI070330 to E.V.S. from the National Institutes of Health (NIH). E.V.S. was additionally supported by NIH grant RO1 CA112021. G.G.O. was additionally supported by the Defense Threat Reduction Agency Medical Biological Defense Research Program, Therapeutic Research Program 4.10007_08_RD_B. K.T. received additional support from NIH grants 1UO1 AI074503 and 1R21 AI077081.

Acknowledgments

Potential conflicts of interest: We declare no commercial interests that might pose a conflict of interest.

The authors thank Enzon Pharmaceuticals, Bridgewater, New Jersey, for providing recombinant human mannose-binding lectin, and members of the Program of Developmental Immunology at Massachusetts General Hospital for insightful comments.

Presented in part: Fourth International Filomeeting, Libreville, Gabon, March 26–28, 2008.

References

1. Shi L, Takahashi K, Dundee J, et al. Mannose-binding lectin-deficient mice are susceptible to infection with Staphylococcus aureus. J Exp Med **2004**; 199:1379–90.
2. Ji X, Olinger GG, Aris S, Chen Y, Gewurz H, Spear GT. Mannose-binding lectin binds to Ebola and Marburg envelope glycoproteins, resulting in blocking of virus interaction with DC-SIGN and complement-mediated virus neutralization. J Gen Virol **2005**; 86:2535–42.
3. Petersen KA, Matthiesen F, Agger T, et al. Phase I safety, tolerability, pharmacokinetic study of recombinant human mannan-binding lectin. J Clin Immunol **2006**; 26:465–75.
4. Valdimarsson H, Vikingsdottir T, Bang P, et al. Human plasma-derived mannose-binding lectin: a phase I safety pharmacokinetic study. Scand J Immunol **2004**; 59:97–102.
5. Bang P, Laursen I, Thornberg K, et al. The pharmacokinetic profile of plasma-derived mannan-binding lectin in healthy adult volunteers patients with Staphylococcus aureus septicaemia. Scand J Infect Dis **2008**; 40:44–8.
6. Brouwer N, Frakking FN, van de Wetering MD, et al. Mannose-binding lectin (MBL) substitution: recovery of opsonic function in vivo lags behind MBL serum levels. J Immunol **2009**; 183:3496–504.
7. Vigerust DJ, Shepherd VL. Virus glycosylation: role in virulence immune interactions. Trends Microbiol **2007**; 15:211–8.
8. Vorup-Jensen T, Sorensen ES, Jensen UB, et al. Recombinant expression of human mannan-binding lectin. Int Immunopharmacol **2001**; 1:677–87.
9. Michelow IC, Dong M, Mungall BA, et al. A novel l-ficolin/mannose-binding lectin chimeric molecule with enhanced activity against Ebola virus. J Biol Chem **2010**; 285:24729–39.
10. Bray M, Davis K, Geisbert T, Schmaljohn C, Huggins J. A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. J Infect Dis **1998**; 178:651–61.
11. Weidmann M, Muhlberger E, Hufert FT. Rapid detection protocol for filoviruses. J Clin Virol **2004**; 30:94–9.
12. Warfield KL, Posten NA, Swenson DL, et al. Filovirus-like particles produced in insect cells: immunogenicity protection in rodents. J Infect Dis **2007**; 196:S421–S429.
13. Clercq ED. Three decades of antiviral drugs. Nat Rev Drug Discov **2007**; 6:941.
14. Zampieri CA, Sullivan NJ, Nabel GJ. Immunopathology of highly virulent pathogens: insights from Ebola virus. Nat Immunol **2007**; 8:1159–64.
15. Baize S, Leroy EM, Georges-Courbot MC, et al. Defective humoral responses extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. Nat Med **1999**; 5:423–6.